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Renal kallikrein-kinin system

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Although the primary function of the kidney is regulation of the volume and ionic composition of body fluids, it is also an endocrine organ with an important role in the regulation of blood pressure. Some renal hormones have both vasoconstrictor and antinatriuretic effects (vasopressor systems) while others have vasodilator and natriuretic effects (vasodepressor systems). A characteristic of these systems is that they act not only on distant target organs but also in the kidney itself (local hormones or autacoids). For example, the renin-angiotensin, prostaglandin, and kallikrein-kinin systems, whose activation and actions appear to be interrelated, may participate in the control of blood pressure not only by altering the tone of extrarenal blood vessels, but also by directly regulating intrarenal sodium and water excretion, perhaps by regulating renal blood flow distribution.

The renal kallikrein-kinin system is the least well defined of these systems, yet it has been implicated in the control of renal blood flow and function, in the pathogenesis of hypertension, Bartter syndrome, and renal diseases, and in the antihypertensive mechanism of drugs such as diuretics and angiotensin I converting enzyme inhibitors. However, the unequivocal role of the renal kallikrein-kinin system in these normal and pathological conditions still remains unknown.

Components of the renal kallikrein-kinin system and their measurement

Kallikreins are serine proteases that release kinins from plasma substrates called kininogens. The two main classifications of kallikrein are plasma and glandular. There are also two main forms of kallikrein substrate—low and high molecular weight kininogens—which are found in plasma [1–4]. Plasma kallikrein, also known as the Fletcher factor, releases kinins only from high molecular weight kininogen, also known as the Fitzgerald factor. Plasma kallikrein is found mainly in the inactive form (prekallikrein), and, together with high molecular weight kininogen and Hageman factor, is involved in the intrinsic blood-clotting mechanism through the activation of factor XI [2]. This system is also involved in the activation of plasminogen to plasmin and in the organism's response to injury and inflammation. It is not known whether plasma kallikrein, through the release of bradykinin, is involved in the regulation of blood pressure and flow. The plasma kallikrein system differs from the glandular kallikrein system in its biochemical, immunological, and functional characteristics. Table 1 demonstrates differences between these two systems.

Glandular kallikreins are found in the kidney, pancreas, intestine, salivary, and sweat glands, and in the exocrine secretions of these organs. We and others have found that there is also immunoreactive glandular kallikrein in plasma [1, 5, 6]. Further, there is evidence that blood kinins may be generated by glandular kallikrein or kininogenases other than plasma kallikrein [7, 8]. In humans [9] and the rabbit, 50% or more of the urinary kallikrein is in the inactive form; in the rat 70% is active [10]. Renal kallikrein releases kinins in vitro from both low and high molecular weight kininogen. However, the natural substrate for this enzyme is probably low molecular weight kininogen since a patient with a congenital deficiency of plasma high molecular weight kininogen (Fitzgerald trait) has normal amounts of kinins in the urine (unpublished data). Renal kallikrein releases kallidin (lys-bradykinin), which is partially converted to bradykinin by an aminopeptidase present in plasma and urine [9]. Rat urinary kallikrein is an exception because it has recently been reported that it released bradykinin [11]. Methionyl-lys-bradykinin is formed by uropepsin when the urine is collected at acidic pH [9]. Kinins are inactivated rapidly by peptidases called kininases that are found in blood and other tissues. The two main kininases have been named kininase I and II. Kininase I is an arginine carboxypeptidase and kininase II, also known as angiotensin I converting enzyme, is a peptidyl dipeptidase [12]. Other kininases have also been identified. Figure 1 shows the structure of bradykinin, lys-bradykinin (kallidin), and met-lys-bradykinin, as well as the cleavage sites from kininogen by plasma and glandular kallikrein, uropepsin, kinins of aminopeptidase, and kininase I and II.

The activity of the renal kallikrein-kinin system is inferred usually from measurements of urinary kallikrein. However, the activity of this system could be regulated not only by the amount of kallikrein secreted in the tubular fluid but also by the kininogen concentration, presence of kallikrein inhibitors, pH and ionic composition of tubular fluid, presence of kininases, and sensitivity of the target organs. In most studies, urinary kallikrein excretion has been the only parameter measured, and the activity of this system has been inferred from this determination. Further, the specificity of some methods used to measure kallikrein is questionable, thus presenting limitations that should be considered when interpreting the information available about the renal kallikrein-kinin system.

Kallikrein can be measured by methods based on different principles; however, it is determined frequently by its esterolytic and amidolytic activity. In these procedures, the

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Table 1. Characteristics of glandular and plasma kallikrein

	Glandular kallikrein	Plasma kallikrein
Molecular weight	24,000–44,000	100,000–120,000
Isoelectric point	3.5–4.4	8.0–8.5
Substrate	LMWK and HMWK	HMWK
Inhibited by SBTI	No	Yes
Normally found as	Active and inactive	Inactive (prekallikrein)

Abbreviations: LMWK, low molecular weight kininogen; HMWK, high molecular weight kininogen; SBTI, soybean trypsin inhibitor.

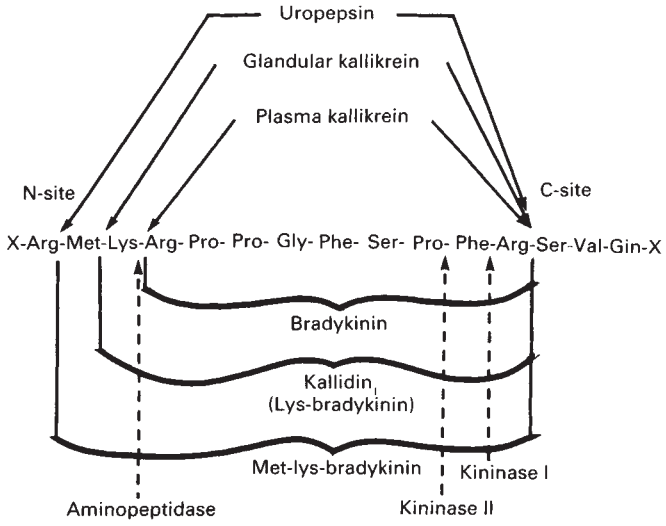

Fig. 1. *Structure of bradkyinin, kallidin, and met-lys-bradykinin. Solid arrows* indicate the sites of kininogen cleavage by kininogenases; *dashed arrows* indicate the site of kinins cleavage by kininases.

enzyme is measured by its capability to cleave the ester or amido bond of synthetic substrates. Although these assays are relatively simple, they lack specificity. Another frequently used method is based on the kininogenase activity of the enzyme. In this method, kallikrein is measured by its capacity to release kinins when it is incubated with kininogen, and generated kinins are measured by either bioassay or radioimmunoassay [13]. Recently, it has been reported that a nonkallikrein urinary esterase (esterase A) that is found in rat urine is also capable of generating kinins; however, its kininogenase activity per esterase unit is much lower than that of kallikrein [1]. A direct radioimmunoassay (RIA) for measuring the kallikrein protein moiety has also been developed. This RIA is highly specific for glandular kallikrein; however, the method measures both active and inactive kallikrein. This RIA has allowed the quantification of circulating immunoreactive glandular kallikrein [5, 14, 15].

The activity of the renal kallikrein-kinin system can also be assessed by measuring kinins that are formed in the renal vascular compartment or by determining urinary kinin excretion since kinins are the biologically active part of this system. However, kinins are catabolized rapidly in the kidney and blood and while the urine is in the bladder since urine contains kininase activity [16]. Thus, renal vein and urinary kinins may not reflect the concentrations reached at the intrarenal site of production.

Origin, subcellular, and cellular localization of the renal kallikrein-kinin system

Origin. Urinary kallikrein is synthesized by the kidney. In vitro incubation of kidney slices or suspension of the renal cortical cells results in the secretion of kallikrein into the incubation medium [17, 18]. When isolated rat kidneys are perfused with fluids that contain neither prekallikrein nor kallikrein, they produce and release active and inactive kallikrein in both the urine and the perfusion fluid. The kallikrein found in the perfusion fluid appears to be secreted directly into the vascular compartment because it is found in the perfusion fluid of nonfiltering kidneys [19].

Recently, we found that plasma immunoreactive glandular kallikrein increased three to four times after nephrectomy, and that its concentration was lower in the renal venous than in arterial blood. These data suggest that the kidney filters and removes plasma immunoreactive glandular kallikrein [15]. Immunoreactive kallikrein has been found in lymph collected from rat kidneys [20]. However, this material was reported to be enzymatically inactive.

A small but significant increase in immunoreactive kallikrein in plasma was found in rats that had received a low sodium diet, which is known to increase renal and urinary kallikrein [21]. However, salt depletion also increases kallikrein in the salivary glands [22]; whether it also enhances its release is unknown. Thus, the source of plasmatic glandular kallikrein is still not clear.

A simultaneous increase in esterase activity in the renal lymph and urine of dogs during infusion of saline has been reported suggesting that active renal kallikrein is released in the interstitial spaces of the kidney [4]. However, as mentioned previously, esterolytic methods are highly unspecific, and findings of this study should be interpreted with caution. The possibility that the kidney releases kallikrein in vivo into the vascular compartment, as shown in the perfused kidney [19], cannot be excluded. Renal kallikrein in the interstitial and vascular spaces of the kidney could release kinins from plasma kininogen, thereby possibly playing a role in the regulation of blood flow distribution. At the present time, it is unclear whether glandular kallikreins released from kallikrein-containing organs reach the systemic circulation in their enzymatically active form. The immunoactive material present in rat plasma was found to be inactive [23]; however, enzymatically active glandular kallikrein has been purified recently from human plasma [6]. We have evidence indicating that kallikrein released from the submaxillary glands into the vascular space can generate kinins in the systemic circulation [8].

There are considerable amounts of protease inhibitors in plasma that could rapidly inactivate serine proteases such as tissue kallikrein. However, the interaction of plasma or tissue protease inhibitors with glandular kallikrein is poorly understood. Alpha$_2$ macroglobulin does not inhibit the enzyme, and alpha$_1$ antitrypsin was reported either to need hours to inhibit it [24] or not to inhibit it at all [25]. In summary, whether renal kallikrein can release kinins either systematically and/or within the renal circulation is still unclear.

Subcellar localization. There is no complete agreement as to the subcellular localization of renal tissue kallikrein. Some investigators have found kallikrein activity associated with the

lysosomal fractions. Others have found that kallikrein was localized mainly in the microsomal fractions [1, 3].

Evidence suggests that kallikrein is present in plasma membranes [26] with the active site facing the cell exterior [27]. Recently, it was reported that kallikrein was present in free polyribosomes, endoplasmic reticulum, and Golgi complex structures, and also in both the lumenal and basal membranes by immunoelectron microscopy [28], although most of the kallikrein was associated with the membranes that face the urinary compartment.

Taken together, these data on the subcellular localization suggest that kallikrein may be synthesized as prekallikrein in the ribosomes attached to the membrane of the endoplasmic reticulum from which it is transferred into cysternal spaces and then to Golgi complex structures where the carbohydrate moiety is added. Then, it is possible that these vesicles either transform into lysosomal-like particles or directly fuse with lysosomes where part of the prekallikrein may undergo selective proteolysis, which results in active kallikrein. Subsequently, the kallikrein-containing vesicles condense with the plasma and basolateral membranes, possibly with the active site of the enzyme facing the urinary compartment (plasma membrane) and the interstitial and vascular space (basolateral membrane). In vitro, membrane-bound kallikrein activity is increased by incubation with phospholipase A_2 and also by incubation with lysolecithin, arachidonic acid, and mellitin [29]. This suggests that activation of phospholipases leads to compounds involved in kallikrein activation. It is possible that there is feedback between phospholipases and kallikrein since glandular kallikrein was shown to be a potent stimulator of prostacyclin release in endothelial cells apparently through phospholipase activation [30].

At the present time, it is unknown whether kallikrein bound to the membranes could release kinins in vivo from either urinary and/or interstitial kininogen without being released into the urinary and interstitial vascular spaces.

Cellular localization. Over 90% of kidney kallikrein is found in the renal cortex, decreasing from the outer to the inner cortex, with very little kallikrein in the medulla and papilla [31]. Isolated glomeruli have a small amount of kallikrein activity compared to the kallikrein concentration in the total cortex. Using the stop-flow technique we have shown that kallikrein is secreted into the urine in the distal tubule [32]. Using immunohistochemical techniques, several studies have shown that kidney kallikrein in rats and humans is localized in the convoluted distal tubules. In these studies, no kallikrein could be found in the collecting tubules or the area of the macula densa [33]. However, we have evidence that kallikrein-containing cells are associated with the juxtaglomerular apparatus in rats (Barajas, personal communication). Simson et al [34] reported a broader distribution of kallikrein in the rat nephron including the proximal tubule, distal tubule, and collecting tubule near the tip of the papilla. In the proximal tubule, kallikrein was observed as reabsorption droplets. Reabsorption droplets were also observed in the proximal tubule of human nephrons. Since the kidney removes immunoreactive kallikrein from plasma, it may be that these reabsorption droplets are immunoreactive fragments of filtered glandular kallikrein that are incorporated in this segment of the nephron by endocytosis.

We recently studied the localization of both active and inactive kallikrein in the microdissected rabbit nephron [35, 36]. We selected the rabbit because in the distal tubule where kallikrein is probably localized, transition from one segment to another is more abrupt than in other species. Active and inactive kallikrein was localized in the granular portion of the distal and cortical collecting tubules. This segment of the nephron contains more than 85% of the active and inactive kallikrein found in the total microdissected nephron. Very little or no active or inactive kallikrein was found in other segments, including the bright portion of the distal segment, which has the macula densa. The granular portions of the distal convoluted and cortical collecting tubules have similar characteristics both morphologically and functionally. They form a single nephron segment called the connecting tubule, which consists of the connecting tubule cells and intercalated cells. Since the connecting tubule cells and kallikrein localized only in the granular part of the distal and collecting segment, and since the intercalated cells are distributed from the granular distal tubule to the medullary collecting tubule, we proposed that kallikrein is synthesized in the connecting tubule cells. Using an antibody produced in our laboratory, Figueroa et al [28] have confirmed recently that rat renal kallikrein is localized in connecting cells of the distal nephron. The discrete localization of kallikrein suggests a specific role of renal kallikrein in association with the function of these distal nephron cells. A low sodium diet greatly increased both active and inactive kallikrein without altering the distribution profile and without changes in the ratio of active to total kallikrein. This suggests that sodium restriction stimulates synthesis and excretion of both active and inactive kallikrein from the connecting cells.

Localization of kinin synthesis in the nephron. Kinins, the peptides released by kallikrein from kininogen, have potent and widespread pharmacological actions. These include vasodilation, contraction of many smooth muscle preparations, increased capillary permeability, lymph production, and production of arachidonic acid metabolites through phospholipase activiation. Kinins are also able to induce changes in the transepithelial movements of electrolytes, increase cyclic AMP, and induce formation of phosphoinostiosides in some cellular systems [37].

The precise role of intrarenally formed kinins is still unknown. Considerable amounts of kinins are present in urine [9]. They appear to be formed in the kidney and urine itself, since pharmacological amounts of kinins injected into the renal artery fail to appear [38]. The proximal tubule is rich in kininases, thus preventing filtered kinins from reaching the distal nephron [39]. Furthermore, Carone et al [40] have shown that when labelled bradykinin was injected into the proximal tubule, it was almost completely destroyed. However, when this peptide was injected into the distal tubule, it appeared almost intact in the urine. This evidence, plus the fact that renal kallikrein is secreted into the urine at the level of the distal tubule, seems to indicate that urinary kinins may be formed in the distal part of the nephron. We have confirmed this possibility by using the stop-flow technique [41]. We found that kinins are formed in the distal nephron with the highest concentration located in the final segment of the distal part of the nephron or even in the renal papilla and pelvis. No evidence of kinin formation was found in the fraction representing the proximal nephron. Freshly voided urine, which has not been in the bladder for a long period,

sometimes generates kinins for a short time, and then the concentration of kinins starts to decrease. At other times, kinin concentration decreases from the moment the urine is voided (unpublished observations). The reason for these changes is that urine contains not only kallikrein but also kininogen and kininases [10]. Therefore, to measure kinins formed in the kidney, it is necessary to collect the urine directly from the ureters and stop the formation and destruction of kinins immediately after collection. Vinci et al [42] reported a lack of correlation between 24-hr urinary kallikrein and kinin excretion; the urine specimens, however, were collected by spontaneous voiding. Thus, kinins could have been destroyed while the urine was in the bladder. Nevertheless, we also found no correlation between kallikrein and kinin excretion in urine collected directly from the ureter of volume-expanded rats and in the urine of human subjects that had not been in the bladder for more than 30 min [43, 44].

We found that acidification of the urine by infusion of sodium sulfate decreased kinin excretion while it increased kallikrein excretion [43]. It is possible that the lower pH within the distal nephron decreases the kininogenase activity of renal kallikrein, because the optimum pH of kallikrein is 8.5. This suggests that changes in the basal urinary pH level influence the urinary excretion of kinins. Recently, Kauker et al [45] reported that the urinary excretion of kinins varied in relation to the urinary level of vasopressin, irrespective of urinary volume, osmolarity, or kininogenase activity. These data again indicate that the intrarenal formation of kinins is determined not only by kallikrein but also by other factors.

When bradykinin is infused into the renal artery, 90% is inactivated in one passage through the kidney and less than 0.25% appears in the urine [38]. Among kininases, kininase II has been studied more extensively than kininase I, partly because kininase II hydrolyzes kinins and also converts angiotensin I to angiotensin II. Kinins could be considered the preferred substrate for kininase II, since the K_m is lower for this peptide than for angiotensin I. Kininase II has been localized in the brush border of the proximal tubule cells of the kidney using fluorescent-labelled antibody to kininase II and also by demonstrating a high concentration of this enzyme in brush bordered membranes that were separated by differential centrifugation [46]. Small concentrations of kininase II were also found in the distal nephron. The presence of kininase II and other peptidases in the lumen of the proximal tubule prevents filtered kinins from reaching the distal nephron, thus acting as an effective barrier [40].

Renal lymph also contains kininase II. A carboxypeptidase distinct from carboxypeptidase A, B, or N has been purified from human urine [47]. Despite the presence of these kinin-inactivating enzymes, large amounts of kinins are found in urine [9]. It could be that kininases are only weakly active at the acidic pH normally found in urine.

The origin of the kininogen (substrate) needed for the formation of kinins in the lumen of the nephron is not known. The plasma of different species are rich in kininogen. The amount is on the order of 4 to 12 μg/ml when expressed as its capability to release kinins when incubated with trypsin. A small amount of plasma kininogen reaching the distal nephron could account for the kinins found in the urine. In human urine, there is a significant amount of immunoreactive kininogen; however,

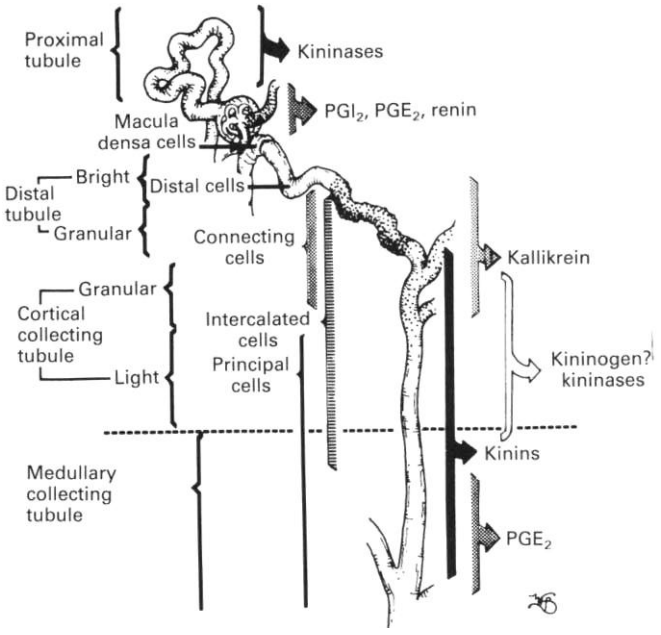

Fig. 2. *Localization of the kallikrein-kinin system, renin, and prostaglandins in the nephron* (right brackets), *anatomical subdivisions of the nephron* (far left brackets), *and type of cells found in the distal nephron(left brackets).*

kininogen is found in only small amounts when measured by its capability to generate kinins [9]. It could be that most of the kininogen has already been consumed by the urinary kallikrein. It has been reported that urinary kinins were absent in a subject who had a congenital deficiency of low and high molecular weight kininogen [9]. On the other hand, as previously mentioned, it was found in a patient who did not have high molecular weight kininogen but who had near normal low molecular weight kininogen (Fitzgerald trait), that the urine contained normal amounts of kinins. This indicates that kinins in the urine are formed from low molecular weight kininogen and that they are not formed by plasma kallikrein, because this enzyme mainly uses high molecular weight kininogen as substrate [3]. Plasma kininogen appears to be synthesized by the liver [3].

Recently, Proud et al [48] isolated kininogen from human urine by immunoaffinity chromatography. Using immunofluorescence techniques with an antibody against the heavy chain of kininogen, Proud et al [48] localized kininogen in the cortical and medullary distal tubules and in the collecting ducts, which suggests that urinary kininogen originates in the distal nephron. However, in the renal tissue of rabbits, we have been unable to demonstrate the presence of kininogen (Omata, Scicli, and Carretero, unpublished results). The presence of an inhibitor of glandular kallikrein has also been demonstrated in the tubules of the rat kidney [49]. It is feasible that this inhibitor could play a role in the regulation of the intrarenal formation of kinins. Figure 2 illustrates the localization of kinin synthesis and degradation in the kidney.

Interrelations between the kallikreinin-kinin system and other hormonal systems. The renal kallikrein-kinin system can activate both the renin-angiotensin and prostaglandin systems. Urinary kallikrein in vitro converts inactive renin to active

renin; a suggestion was made that it could also activate renin in vivo. However, urinary kallikrein appears to hydrolyze only inactive renin that has been exposed previously to acidic pH [50, 51].

There is evidence indicating that both urinary kallikrein and kinins can stimulate release of renin. Suzuki et al [52] reported that urinary kallikrein stimulates release of renin from superfused isolated kidney slices. Neither bradykinin in high doses nor trypsin (a potent kininogenase) was able to release renin under these experimental conditions, indicating that kallikrein was acting by mechanisms other than the generation of kinin. The effect of urinary kallikrein on renin release was blocked by aprotinin, a nonspecific protease inhibitor and amiloride, which in high concentration is also able to inhibit kallikrein [30]. The effect was not blocked by indomethacin or propanolol. The same laboratory has recently re-examined these data. They suggest that the previous observation was not due to renin stimulation but rather to the higher recovery of renin after freezing and thawing in the samples that contained proteins [53]. It is not clear, however, why aprotinin, a polypeptide, or amiloride, a low molecular weight organic compound, could prevent the protective effect of proteins on renin activity. The effects of glandular kallikrein on renin release were further studied by our laboratory [54]. We found that either hog or rat submandibular kallikrein can stimulate renin release from isolated superfused rat glomeruli. This effect could not be explained by unspecific protection of renin because it occurred in the presence of 0.1% BSA and was abolished by treatment of kallikrein with aprotinin or phenylmethylsulphonylfluoride (a serine protease inhibitor). These data leave open the possibility that kallikrein, or a closely related enzyme, plays a role in the activation of renin release; however, the physiological significance of the in vitro effects of kallikrein on renin release are unclear at this time. In vivo, bradykinin infused into the renal artery of dogs stimulates renin release [54]. In vitro kinins increased renin release from isolated superfused glomeruli [55]. It may be that Suzuki et al [52] did not observe similar effects with kidney slices because kininases degraded kinins rapidly.

Aprotinin, an unspecific serine protease inhibitor, inhibited renin release in humans and furosemide-stimulated renin release in rats and rabbits [56–58]. It is still unclear whether these effects of aprotinin are due to inhibition of glandular kallikrein or other serine proteases or are unrelated to its inhibitory activity. Additional studies using different kallikrein or protease inhibitors are needed to clarify this point.

The angiotensin I converting enzyme (kininase II) further links the kallikrein-kinin and renin-angiotensin systems [12]. The converting enzyme is found in high concentrations on the endothelial cell surface of the lung's vascular bed and has the concurrent functions of converting angiotensin I to II and destroying kinins. There is evidence that over 90% of the kinins administered in the venous side of the systemic circulation are destroyed by one passage through the lung, which suggest that most of the intrarenally formed kinins that might enter the renal venous blood would be destroyed before they reach the peripheral circulation. This suggests that kinins formed intrarenally act as vasodilators within the confines of the renal vasculature, perhaps regulating blood flow to specific areas in the nephron.

Mineralocorticoid administration increases urinary kallikrein excretion and renal kallikrein concentration while spirolactone, an aldosterone antagonist, decreases it [1, 3, 4, 18]. These findings suggest that renal kallikrein is regulated by mineralocorticoids. However, the increase in kallikrein occurs in vivo after the mineralocorticoids induced changes in renal function. In dogs treated with deoxycorticosterone acetate (DOCA) kallikrein increased after the dogs had escaped from the sodium-retaining effects of the steroid [60]. In microdissected nephron segments from rabbits the transepithelial voltage of the connecting tubule where kallikrein is located is independent of DOCA, while that of the light portion of the cortical collecting tubule is affected by mineralocorticoids [1]. It was reported recently that adrenalectomy decreased the content of both active and inactive kallikrein in connecting tubules as well as that of Na-K-ATPase in cortical and medullary collecting tubules [61]. After aldosterone injections, Na-K-ATPase returned to normal values while the content of kallikrein remained depressed. Thus, kallikrein does not appear to be regulated directly by mineralocorticoids. We feel that the accumulated data suggest an indirect action of corticosteroid hormones on the synthesis and release of renal kallikrein, perhaps through an alteration of water and electrolyte metabolism.

Kinins infused into the renal artery stimulate the synthesis of prostaglandins, probably prostaglandin E_2 (PGE_2), in the collecting duct and renal medulla and prostacyclin (PGI_2) in the arterioles [1, 3, 4].

This effect of kinins is the result of acylhydrolase stimulation, which leads to arachidonic acid release from phospholipids, and thereby provides substrate for the formation of cyclooxygenase and lypooxygenase products. Several studies suggested that some of the effects of kinins in renal function relate to concomitant prostaglandin generation. It was reported that part of the renal vasodilator and natriuretic effects of kinins can be inhibited by cyclooxygenase inhibitors. However, these findings have not been confirmed in subsequent studies [62]. Kinins generated intrarenally appeared to stimulate release of a PGE-like substance from an isolated rabbit kidney that was perfused with Krebs' solution containing kininogen. Kinins induced the release of various prostanoids including PGE_2 and 6-keto-$PGF_{1\alpha}$ from rabbit renal papillary collecting cells in culture [63]. Kinins are also able to stimulate prostaglandin synthesis from rat kidney mesangial cells [64]. Monolayers of canine cortical collecting tubule cells responded to bradykinin stimulation with prostaglandin release only when the kinins were added to the apical surface, which suggeted that urinary but not blood-borne kinins elicit prostaglandin formation in these cells [65]. Interestingly, prostaglandins were released from both the apical and basolateral surface. Prostaglandins have been reported to stimulate urinary kallikrein excretion [4], while phospholipase A2 can activate membrane-bound renal kallikrein [26]. Mineralocorticoids can increase urinary kallikrein and PGE_2 simultaneously. The increased excretion of PGE_2 in DOCA-treated rats was found to be decreased by administration of aprotinin, an unspecific serine protease inhibitor [66]. Prostaglandins stimulate, while cyclooxygenase inhibitors suppress, kallikrein release [62]. Angiotensin, another prostaglandin releasing agent, has also reported to stimulate renal kallikrein excretion [4].

These data suggest that kinins formed in the distal nephron may stimulate production of prostaglandins, particularly by the

cells of the collecting duct, interstitial cells of the renal medulla, and endothelial cells of blood vessels. Kallikrein by itself appears to be a potent stimulator of PGI_2 release from endothelial cells in culture [30]. Kallikrein release and prostaglandin production may be linked under circumstances not yet well defined.

The interactions between the kallikrein-kinin system, renin angiotensin-aldosterone, and prostaglandin systems may play an important physiological role. It could be speculated that an increase in renin-angiotensin system activity would produce both a peripheral and renal vasoconstriction that could impair renal blood flow. However, angiotensin II and aldosterone stimulates the release of renal kallikrein and prostaglandins, which could produce local vasodilation and maintain renal blood flow.

Antidiuretic hormones may also be linked to the renal kallikrein-kinin system. Antidiuretic hormone (ADH) stimulates both the release of kallikrein and intrarenal formation of kinins [67]. Bradykinin acts only from the basolateral surface of rabbit collecting tubules to antagonize the hydrosmotic effects of ADH; this effect appears to be mediated through prostaglandins [68]. It is possible that intrarenally formed kinins antagonize or modulate the effects of ADH in the kidney either directly or through the release of prostaglandins.

In conclusion, there are numerous complex interactions among the kallikrein-kinin, renin-angiotensin-aldosterone, ADH, and prostaglandin systems of the kdiney. Many are not completely understood. Some may be of no physiological importance. In these systems abnormalities have been observed that suggest that the interaction of kinins, renin, and prostaglandins may play an important role in the regulation of renal function in physiological and pathological situations.

Physiological role of the renal kallikrein-kinin system

Although some of the actions of renal kallikrein may be due to its catalytic effect on substrates other than kininogen, most of its effects seem to be mediated by kinin release.

The evidence indicating a role for the renal kallikrein-kinin system in the regulation of renal vascular resistance is mostly indirect. Infusion of kinins into the renal artery increases renal blood flow, diuresis, and natriuresis. The increase in blood flow is greater in the outer than in the inner cortex [1]. These effects on renal function are common to most vasodilators and by themselves only suggest that the kidney has receptors for kinins. It is unlikely that arterial injections of kinins mimic the effects of kinins endogenously generated by renal kallikrein, since the sites of action, the concentration, or the sequence of vascular elements affected may be different. It has been reported that administration of inhibitors of kininase II (such as captopril), induces increase in the renal blood flow and of renal venous blood and urinary kinins [1]. Thus, it is possible that part of these effects are due to an increase in the intrarenal concentration of kinins. However, these types of studies do not discriminate between the effects that result from kinins from those that result from the inhibition of conversion of angiotensin I to the powerful vasoconstrictor angiotensin II. In sodium-depleted dogs, it is likely, for example, that the changes induced by kininase II inhibitors are due to lowering of angiotensin II since the infusion of an angiotensin antagonist produced the same changes [3]. However, Clappison et al [69] found that dogs in which the renin-angiotensin system was inhibited by pretreatment with DOCA and a high salt diet, or by simultaneous infusion of an angiotensin antagonist, still responded to kininase inhibitors with the same qualitative changes in renal blood flow. Another recent study showed that sodium-depleted rats did not have an increase in renal blood flow when they received infusion of kinins. The response to kinins was, however, restored if the rats were administered pretreatment with aprotinin. In addition, rats on a high sodium diet responded normally to kinins infusion. In these sodium-repleted rats, the renal vascular resistance was decreased by a kininase II inhibitor, but it was not affected by saralasin, an angiotensin antagonist. Interestingly, the action on renal blood flow of captopril was blocked by aprotinin [70]. These data were interpreted as suggesting that the influence of the kallikrein-kinin system upon renal vascular resistance was maximal during sodium depletion and, also, that the increase in renal blood flow induced by captopril when on a high sodium was due to a decrease in the inactivation of kinins. Again these studies do not differentiate between kinins generated locally by renal kallikrein or systemically by plasma kallikrein. However, aprotinin is a poor inhibitor of plasma kallikrein [2]. To influence renal vascular resistance, kallikrein should be released into the basolateral membrane side and generate kinins, which then should gain access to the vascular smooth muscle of the glomerular arterioles. The evidence for this is conflicting. As described before, Rabito et al [15] found that there is less immunoreactive glandular kallikrein in arterial plasma than in renal vein plasma. No evidence of increased kallikrein in renal lymph was found by Proud et al [20]. These data do not favor the notion of a passage of renal kallikrein into the interstitial space. However, this possibility cannot be discarded. Isolated perfused kidneys release kallikrein into the perfusate, thus indicating release into the interstitial space [13, 19]. Whether this finding can be extrapolated to an in vivo situation is not as yet clear. Anatomically, release into the basolateral side is possible since kallikrein has been found in basolateral membranes [29]. Using electronimmunocytochemistry, Figueroa et al [28] recently showed that a small percentage of the kallikrein present in connecting cells of the distal nephron appears to be incorporated to and released from the basolateral membrane. Studies with specific inhibitors of glandular kallikrein may have to be performed before the role of the renal kallikrein-kinins system in regulation of renal vascular resistance can be clearly understood.

Unlike other vasodilators, kinins do not induce diuresis and natriuresis by decreasing proximal sodium and water reabsorption in outer nephrons, as determined by micropuncture [1, 3]. In consequence, kinin-induced natriuresis is either due to inhibition of sodium reabsorption in the distal part of the nephron or in deep proximal nephrons not available for micropuncture. Kinins may affect sodium reabsorption as a result of a direct effect on sodium reabsorption in the nephron or because of changes in the osmotic gradient in the renal medulla or both. Changes in the osmotic gradient of the renal medulla could explain the decrease in urinary osmolarity and vasopressin-resistant diuresis caused by kinins [1, 3]. The medullary osmotic gradient may have been changed by the increased blood flow brought about by the peptide and/or by decreased reabsorption from the distal nephron. To our knowledge, studies on changes in medullary blood flow during kinin administration have not been reported.

As noted before, kinins administered intrarenally probably do not mimic the effects produced by kinins formed intrarenally since their site of action or concentration could be different. However, a study conducted by Kauker [71] suggests that lumenal kinins could indeed be natriuretic. He has shown that kinins administered in the late proximal nephron could double the excretion of ^{22}Na, which was simultaneously perfused into the tubule [71]. This study should be interpreted with caution, however. The enhancement by kinins of sodium excretion could have occurred proximal to the distal and collecting tubules where kinins are formed. Normally, kininases present in the proximal nephron would presumably metabolize any filtered kinins. Thus, the effects of kinins are expected to occur either at the site of production or downstream from it. In addition, Kauker [71] used only one concentration of kinins (100 pg/nl), which is much higher than the concentration that can be found in the nephron, since a rat kidney excretes less than 1000 pg of kinins/ml urine during water diuresis [43]. Nevertheless, these data indicate that decreased sodium reabsorption occurs when kinins are present within the lumen, at least under the conditions used by Kauker [71]. This is also suggested by other circumstantial evidence; the administration of aprotinin, an inhibitor of proteases, to volume-expanded rats decreased glomerular filtration rate, renal blood flow, urinary sodium, potassium and PGE_2. Furthermore, the administration of antibodies against kinins to saline-expanded rats has been reported to result in a decrease in sodium excretion [1, 3, 4, 72]. These findings indicate that endogenously released kinins cause natriuresis, diuresis, and also intrarenal release of prostanoids. Again, however, these types of studies do not clearly distinguish between kinins generated by either plasma or renal kallikrein. Kinins applied to the lumenal side of isolated rat colon produced no changes in either sodium fluxes or short-circuit current. In contrast, large increases in short-circuit current were observed when kinins were applied to the antilumenal side, due principally to augmented chloride movement toward the lumen of the colon [73]. These effects were attenuated by the administration of furosemide and a cyclooxygenase inhibitor. These studies were performed with a concentration of kinins (1 μM), which was 500.000-fold lower than that used by Kauker [71] and is presumably a physiological concentration. These data do not suggest that lumenal kinins affect either Na or Cl movement, at least in the colon. It is of interest that the antagonist action of kinins over vasopressin in isolated rabbit tubules was also exclusively seen when applied to the antilumenal side and appeared to be prostaglandin-dependent [68]. However, aprotinin and 1D-phe-D-phe-L-arg-chloromethyl ketone, which are kallikrein inhibitors, reduce to a greater extent short-circuit current in the bladder of toads when they are on the mucosal side [3], presumably by inhibiting a kallikrein-like enzyme. All these data, including those obtained in the rat colon, suggest that kinins generated intrarenally either on the basolateral side and/or the lumen of the distal nephron are saluretic.

If one assumes that changes in the intrarenal formation of kinins are reflected by changes in the excretion of kallikrein, then the known data are much more controversial. However, this assumption has never been substantiated. In fact, our studies and those of others show that measuring the urinary excretion of kallkrein does not reflect the intrarenal formation of kinins [1, 9, 43, 44]. This should be expected since the concentration of the enzyme in urine is much higher than that of substrate [10]. Further, variations in urinary pH, ionic concentration of the urine, kininases, and the possible presence of inhibitors indicate that kinins released by kallikrein within the kidney may not be reflected by kallikrein measurements.

Urinary kallikrein excretion has been found to correlate inversely with dietary salt intake while high potassium intake has been shown to increase urinary kallikrein excretion [3]. These effects may be secondary to an increase in aldosterone secretion. High kallikrein excretion has been noted in most but not all patients who have primary aldosteronism and in those who have Bartter syndrome; these are conditions characterized by high aldosterone secretion [1, 74]. However, high aldosterone secretion is not always accompanied by high kallikrein excretion. Rats that have 2-kidney, 1-clip renovascular hypertension have normal or high plasma aldosterone concentrations, low kallikrein excretion, and low renal tissue kallikrein concentrations. Furthermore, an excess of mineralocorticoids does not always produce high kallikrein excretion [1, 3, 4, 75]. Therefore, other still unidentified factors control renal kallikrein excretion.

In humans, dexamethasone and ACTH also significantly augment the excretion of kallikrein [76, 77]. Hypertensive doses of dexamethasone resulted in suppression of urinary kallikrein [78].

Water loading has been found to increase kallikrein excretion; however, this finding is not universal [1, 3, 4]. Diuretics increase urinary kallikrein excretion, an effect that appears to be independent of their site of action in the nephron [3]. Acetazolamide, which acts in the proximal tubule, furosemide and bumetanide, which act on Henle's loop, and thiazides, which act on the distal tubule, increase kallikrein excretion. The increase in urinary kallikrein excretion significantly correlates with the excretion of sodium, potassium, and water. Because diuretics stimulate the secretion of aldosterone, it may be assumed that their effect on kallikrein excretion is mediated by this mineralocorticoid. However, diuretics stimulate kallikrein excretion within a few minutes of administration, while aldosterone increases kallikrein excretion only after 3 or more days of administration. Thus, aldosterone does not mediate the acute effects of diuretics. It could be that part of the natriuretic, diuretic, and antihypertensive effects of diuretics is due to increased kinin formation. However, it has been reported recently that amiloride, a diuretic that inhibits sodium entrance into the distal tubule cell from the lumenal side, inhibits the enzymatic activity of urinary kallikrein in vitro [30]. We found that the excretion of kallikrein and of kinins were depressed after acute administration of amiloride [43]. Thus, the role of the kallikrein-kinin system in the mechanisms of action of diuretics needs further definition. The modification of kallikrein release induced by diuretics suggest a link between the cellular changes induced by diuretics and the mechanisms controlling kallikrein release. However, these diuretic-induced changes may be an epiphenomenon associated to the augmented urinary volume and electrolyte excretion induced by the drugs.

In conclusion, the above-mentioned data suggest that the kallikrein-kinin system is involved in the regulation of electrolyte and water transport by the distal part of the nephron. These effects could be due either to a direct effect of kinins on sodium

transport in the distal nephron, to changes in renal blood flow distribution, or to both.

Renal kallikrein-kinin system in human and experimental hypertension

The theoretical possibility that hypertension results from either an excess of vasopressor substances or from a deficiency of vasodepressor substances has stimulated research on the role of the renal kallikrein-kinin system in the pathogenesis of various types of human and experimental hypertension. Furthermore, the kidney, which is considered one of the most important determinants in the long-range control of blood pressure, has both vasopressor (renin-angiotensin system) and vasodepressor (kallikrein-kinin and arachidonic acid metabolites) components. These vasoactive substances could also participate in the regulatory capacity of the kidney to excrete sodium and water.

There are many reports indicating that urinary kallikrein excretion is decreased in patients who have essential hypertension [1, 3, 4]. However, there are indications that if variables such as renal function and race are considered, urinary kallikrein excretion in hypertensive and control subjects is comparable [79]. Persons of the black race have lower kallikrein levels than white persons [79], and patients who have low creatinine clearance have depressed levels of kallikrein [80, 81].

However, we and others [1, 3, 82–84] have reported that kallikrein excretion is decreased in patients who have essential hypertension and normal renal function when compared with normotensive control subjects who have been matched for age, race, and sex. The reason for the discrepancy among these studies is unknown. It is interesting to note that many hypertensive patients have normal kallikrein excretion whereas others have conspicuously low amounts of kallikrein.

Most but not all patients who have primary aldosteronism have increased kallikrein excretion while those who have renovascular hypertension have decreasd kallikrein excretion, despite secondary aldosteronism [1, 3, 4]. These data indicate once again that although mineralocorticoid activity is an important regulator of renal kallikrein, further regulatory mechanisms exist.

In an epidemiological study in which urinary kallikrein concentration (esterase activity) was measured in a large population of healthy children and their mothers, there was a significant familial clustering of urinary kallikrein excretion [84]. Urinary kallikrein concentration was significantly lower in black than in white children. It was altered by season (lower in summer) and by time of day (highest in morning). Families with the lowest mean kallikrein concentrations tended to have higher blood pressure than families with the highest kallikrein concentrations, suggesting concomitant and genetic influence on both blood pressure and kallikrein excretion. This study involved more than 600 children and 163 families; measurements were made in spot urine samples. Uchiyama et al [85] recently reported in a brief communication that no relationship between blood pressure and urinary kallikrein was found in a study involving 77 healthy Japanese children. These conflicting results indicate that the genetic relationship between blood pressure and renal kallikrein has not yet been elucidated.

In this regard, it is interesting to note that urinary kallikrein excretion is decreased in three different models of genetically hypertensive rats developed by selective inbreeding on the basis of their blood pressure [86–89]. Perhaps one of the genetic loci, which controls blood pressure, is linked to one that controls renal kallikrein. At the present, it is unclear whether these are concomitant phenomena, functionally unrelated to each other, or whether the decrease in kallikrein excretion is a pathogenetic factor in the development of hypertension. Another possibility is that the kallikrein excretion is decreased secondary to the increase in blood pressure; however, decreased urinary kallikrein is seen in normotensive children of patients who have essential hypertension and also in rats of the New Zealand genetically hypertensive strain of rats, and the fawn-hooded spontaneous hypertensive rats [1, 3, 89] before they develop hypertension. Urinary kallikrein excretion is decreased in rats bred to be susceptible to the hypertensive effects of salt (Dahl salt-sensitive rats) [14]. Thus, it is possible that the decreased activity of the kallikrein-kinin system resulted in decreased capacity to eliminate excess water and sodium, hence leading to hypertension. However, Dahl's rats have proteinuria when they receive a high salt diet. Rats with proteinuria have higher concentrations of protease inhibitors in their urine. It has been suggested that lower kallikrein in the Dahl rats is due not to decreased synthesis but rather to increased inhibition. When salt-resistant and salt-sensitive rats are raised on a low sodium diet both strains have similar blood pressure and kallikrein excretion [90]. Urinary kallikrein is not always found decreased in genetic experimental hypertension. Stroke-prone spontaneous hypertensive rats and genetically hypertensive mice have increased kallikrein when compared with their respective controls [3].

It is possible that kallikrein excretion is lower when there is an underlying abnormality in renal function, but not when the kidney is uninvolved in the pathogenesis of the hypertension. Lower activity of the kallikrein-kinin system may contribute to decreased capacity to excrete a sodium load in some patients who have essential hypertension and low kallikrein. In addition, decreased kallikrein in urine may not by itself cause abnormal renal function, but it may indicate that the normal mechanisms of synthesis and release of proteins from the distal nephron are altered.

As in humans, rats that have renovascular hypertension have decreased kallikrein in renal tissue and in urine [88, 75]. In two kidney-one clip Goldblatt hypertensive rats, urinary kallikrein was decreased only in urine from the stenotic kidney, while that of the contralateral kidney was normal [91]. It may be that the decrease in urinary kallikrein is related to decreases in renal blood flow as suggested by studies in humans and dogs [3, 4, 79]. Thus, there is coincidence between the general trend observed in human patients and the response in the experimental models. Overlack et al [92] showed that patients who have essential hypertension and low amounts of kallikrein responded to the oral administration of hog glandular kallikrein with a significant fall in the blood pressure. However, this study needs to be confirmed.

In conclusion, urinary kallikrein decreases frequently in patients and animals with primary or secondary hypertension, with the exception of mineralocorticoid-induced hypertension where it is increased or, in some cases, normal. It is not yet clear whether abnormalities in the excretion of kallikrein reflect similar changes in the intrarenal formation of kinins. Reliable

measurements of urinary kinins in patients and experimental animals are scarce. In any case, it has not been proved that a depressed kallikrein-kinin system may have a pathogenetic role in hypertension.

Renal disease and related conditions

Urinary kallikrein excretion is significantly decreased in rats with acute renal failure produced by methemoglobin injection [1]. Kallikrein excretion is also low in other experimental models of acute renal failure [1, 3, 4]. In patients who have chronic renal failure, kallikrein excretion is normal or decreased [81]. However, kallikrein excretions increased greatly when expressed per milliliter of glomerular filtration rate. In patients who have acute tubular necrosis of the vasomotor type, kallikrein reached its highest peak just prior to the resolution of the oliguria [93]. An increase in kallikrein activity per nephron may help in the maintenance, or promotion of better excretory functions and/or blood flow, in the injured kidney.

There are few reports on kallikrein excretion in renal transplant subjects. In humans and dogs, kallikrein is decreased and it has been reported that it decreases even further during rejection [1, 3, 4]. Brouhard et al [94] found that urinary kallikrein increased just prior to the rejection episode. It has also been claimed that amounts of kallikrein are lower in hypertensive than in normotensive graft recipients [95]. These studies should be interpreted with caution since the methodology used to measure kallikrein (esterase activity) is not specific for this enzyme. Recently, in a sutdy in which kallikrein was measured by radioimmunoassay, it was confirmed that kallikrein excretion in graft recipients is lower than in control subjects, but no differences were observed before and during rejection or in relation to blood pressure. Amidolytic and esterolytic activity was observed to increase prior to rejection but immunoreactive kallikrein did not change [96]. Depressed urinary kallikrein may be related to the diminished functional tubular mass of these patients. Esterases other than kallikrein may appear in urine prior to rejection, although more information is needed before elucidating this point. Patients who had cirrhosis without renal failure showed significantly higher amounts of kallikrein than healthy subjects, perhaps because of concomitant secondary hyperaldosteronism. In contrast, patients who have cirrhosis with renal failure had much lower kallikrein excretion than those with maintained renal function, although plasma renin activity and aldosterone are very high [97]. It could be that the kallikrein-kinin system is involved in the maintenance of renal function and blood flow in these patients. Impaired production of kallikrein may contribute to renal failure. Alternatively, decreased kallikrein may reflect impaired tubular function and compromised renal blood flow.

Urinary kallikrein excretion is decreased in the early phase of experimental nephritis that has been induced by antiglomerular basement membrane antibodies and in nephrosis that had been induced aminonucleosides [98]. In antiglomerular basement membrane nephritis, the fall in kallikrein excretion occurs within the first 24 hr concurrent with the onset of proteinuria. In aminonucleoside nephrosis, the decrease precedes the onset of proteinuria by 48 hr, beginning 24 hr after aminonucleoside injection. In experimental chronic glomerulonephritis in rats, kallikrein excretion is normal [99].

It is conceivable that the decrease in kallikrein excretion frequently observed in different acute renal diseases participates in the pathogenesis of these diseases. However, this contention needs further support.

Summary

In the last decade, our knowledge of the renal kallikrein-kinin system has been advanced significantly. More specific and sensitive methods for assessing its activity have been developed. Further, it has been found that in the kidney this system is localized in the distal nephron, where it appears to be linked to processes that control water and electrolyte excretion. Data indicate that the kallikrein-kinin system interacts with other renal hormonal systems such as the prostaglandin and renin-angiotension-kinin system may participate in the control of renal function and the pathophysiology of renal diseases. An increase in kallikrein excretion has been observed after administration of antihypertensive drugs. The kallikrein-kinin system may therefore participate in their mechanism(s) of action.

Our current knowledge suggests that the renal kallikrein-kinin system is an integral part of the intrarenal hormonal system that controls water and electrolyte excretion and participates in the regulation of blood pressure.

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